Solvent Effect on the Interaction of Steroids with a Novel Methyl β-Cyclodextrin Polymer

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ABSTRACT: Methyl β-cyclodextrin was modified by coupling 2-hydroxyethyl methacrylate using hexamethylene diisocyanate as the coupling agent. The methyl β-cyclodextrin-coupled monomer was polymerized thermally using azobisisobutyronitrile as the initiator. The new polymer is insoluble in water and organic solvents. The interaction of the polymer with two typical steroids, namely, cholesterol and testosterone, in different media of varying polarities was tested. The results showed that the polymer has a higher affinity toward cholesterol and the extent of absorption of cholesterol is found to increase with the polarity of the medium. The new polymer can be optimized as a reusable absorbent for steroids, particularly for cholesterol. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 1857–1861, 1998

Key words: hema; methyl β-cyclodextrin; hexamethylene; diisocyanate

INTRODUCTION

Cyclodextrins, the oligomers of D-glucose, have been subjected to extensive studies. The substantial interest on these molecules is due to their ability to form inclusion complexes with a wide class of compounds.[1,2] Today, cyclodextrins (CDs) are predominantly used in drug formulations, in the food industry, and for the separation of isomers and analogs in chromatography.[3–5]

β-Cyclodextrin (BCD), one of the members of the CD family with seven glucose units, has figured in several studies. BCD is well known for its ability to form inclusion complexes with steroids.[6,7] In fact, BCD has been used for the removal of cholesterol from food articles like eggs and butter.

Chemical modifications of BCD have been shown to improve its complexing ability as well as its specificity in complexation.[8] Methyl BCD is a well-known example of modified BCD having interesting properties. The compound has a high affinity toward cholesterol. However, the increased solubility of methyl BCD in water as well as in other common organic solvents curtail its use as a specific reusable adsorbent for cholesterol and other steroids. An insoluble polymer based on methyl BCD may be an ideal absorbent for the removal of cholesterol and other steroids from a medium of interest. Coupling of a vinyl monomer to methyl BCD through one of its —OH groups would be the easier approach to synthesize the polymer. This article addresses the modification of methyl BCD by coupling 2-hydroxyethyl methacrylate (HEMA) using hexamethylene diisocyanate (HMDI) and the subsequent thermal polymerization of the modified methyl BCD. The preliminary evaluation of the methyl BCD-based polymer as an absorbent for cholesterol as well as the influence of polarity of the medium on the interaction with steroids are also discussed.

EXPERIMENTAL

Methyl β-cyclodextrin (BCD), cholesterol, and testosterone, obtained from Sigma Chemical Co.

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(St. Louis, MO) were used as received. 2-Hydroxyethyl methacrylate (HEMA) and hexamethylene diisocyanate (HMDI) were obtained from Fluka (Buchs, Germany). Other reagents, either of analytical grade or chromatographic grade, were procured from Spectro Chem (Bombay, India).

Instrumental

Infrared spectra were recorded using a Nicolet (Madison, WI, USA) Impact 410 FT infrared spectrophotometer. The number of scans were 50. Molecular weight was estimated using a Waters Associates (Milford, MA, USA) chromatographic system consisting of a Model 510 solvent delivery pump and a Reynodyne Model 7725 I injector and Model 401 refractive index detector. For estimating the molecular weight, a Waters styragel HR column was used in conjunction with tetrahydrofuran as the mobile phase at a flow rate of 1 mL/min. The column was calibrated using polystyrene standards.

Synthesis

The synthesis of a BCD-based polymer was reported earlier.[9] A similar procedure was adopted for the synthesis of the methyl BCD polymer. As in the previous case, the molar concentration was chosen in such a way to react only one —OH group of the methyl BCD. We presumed equal probability for all the —OH groups of methyl BCD to participate in the reaction. The stoichiometry in this case was 0.5*M* HEMA:1*M* HMDI: 0.5*M* methyl BCD. HMDI and HEMA were mixed in 25 mL chloroform (ethanol removed by distillation). Dibutyltin dilaurate (catalyst), 0.1%, was added and stirred magnetically at an elevated temperature (45 ± 1°C) under a blanket of nitrogen. The stirring was continued for 1 h. At this stage, the solution was subjected to infrared spectroscopic analysis. A few drops of the solution was placed on a sodium chloride window and chloroform was removed by blowing nitrogen. The spectrum was scanned from 4000 to 600 cm^{-1}. A calculated amount of methyl BCD was dissolved in 10 mL chloroform and then added to the solution. The solution was then stirred at 45°C for nearly 2 h. The syrupy solution was subjected to infrared spectroscopic and gel permeation chromatographic analysis.

Azobisisobutyronitrile, 0.3%, was added to this solution and heated to 60–65°C. The polymerized product was washed extensively using chloroform and then methanol. The product was dried and powdered.

Interaction of the Polymer with the Steroids

Cholesterol and testosterone were used as typical steroids. Solutions of these two compounds were prepared in methanol and hexane. About 60–70 mg of the polymer was placed in the solutions at room temperature (31 ± 1°C) under a static condition. After 1 h, the polymer was collected from the respective solutions by filtration and then transferred to conical flasks. Methanol, 10 mL, was added to each of the flasks and heated to 50°C for 30 min to extract the absorbed steroids. The desorbed steroids were estimated using a high-performance liquid chromatographic procedure.[10] All the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Figure 1 illustrates the infrared spectrum of the product (A) formed between HEMA and HMDI. The —OH stretching band of HEMA is absent in the spectrum, reflecting the reaction between the —OH group and —NCO groups. A peak centered around 3300 cm^{-1} could be assigned to the NH group formed by the reaction. The prominent feature of the spectrum is the peak around 2200 cm^{-1} characteristic of —NCO groups, indicating the presence of residual —NCO groups in the product. An additional weak peak around 1630 cm^{-1} can also be seen which could be assigned to the C=C group of HEMA.

Figure 2 shows the spectrum of the product obtained after reacting A with methyl BCD. A relatively strong peak centered at 3500 cm^{-1} associated with the unreacted —OH group of methyl BCD can apparently be seen in the spectrum. It seems that the 3300 cm^{-1} band (see Fig. 1) is merged with the 3500 cm^{-1} band of the —OH groups. A striking feature of the spectrum is the complete disappearance of the —NCO groups centered around 2200 cm^{-1} (see Fig. 1), reflecting the reaction of —NCO groups with methyl BCD. A strong peak around 1050 cm^{-1} can be seen in the spectrum, which is the characteristic feature of CDs. The spectrum depicted in Figure 3 is that of methyl BCD. A comparison of Figure 2 with that of Figure 3 clearly shows that methyl BCD is reacted with A.

The molecular weight of the product obtained by reacting A and methyl BCD (product B) is

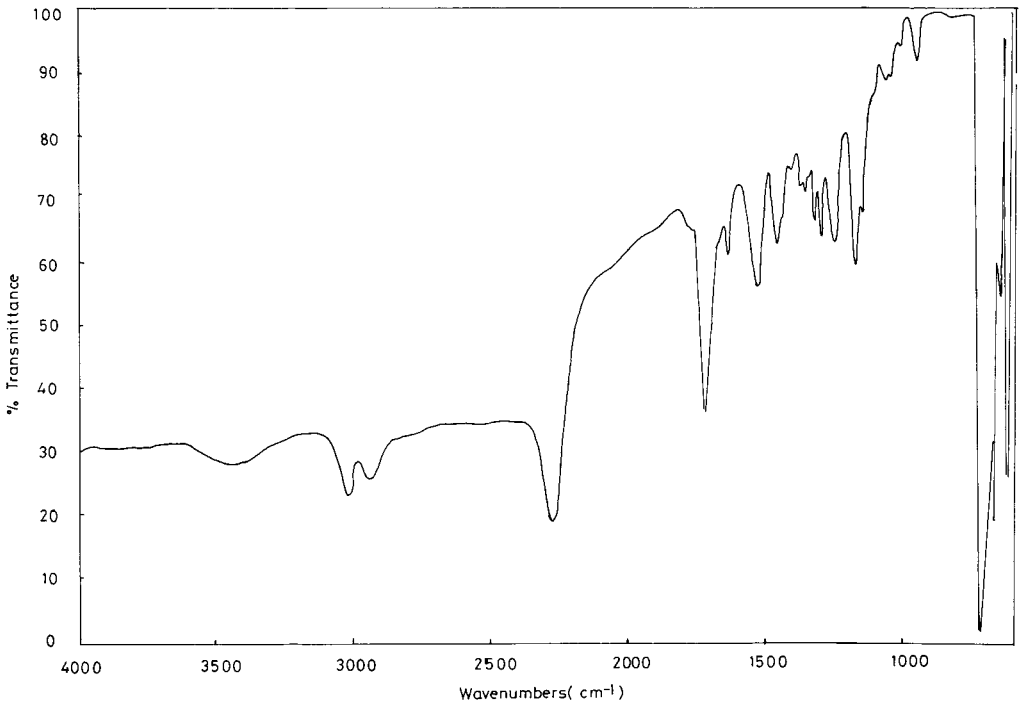

Figure 1 Infrared spectrum of the product obtained after reacting with HMDI and HEMA (A).

1660, which closely agrees with the theoretical molecular weight of 1608 [as per the molecular formula shown in reaction (1)]. Based on the spectral results and molecular weight data, it is quite reasonable to assume that methyl BCD is reacted with A to form

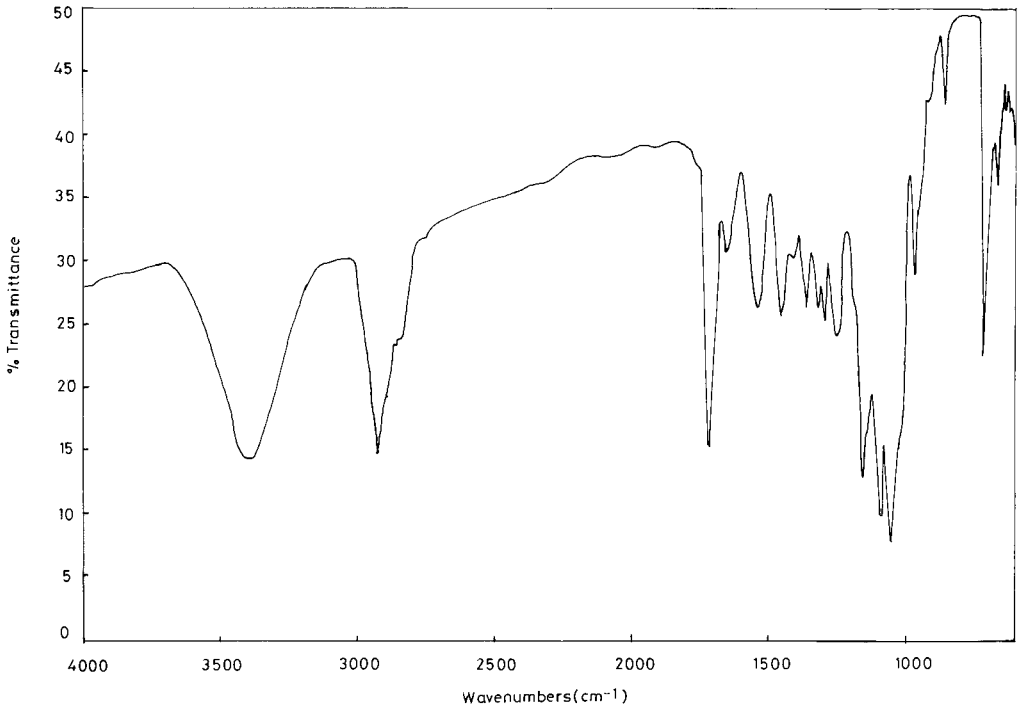

Figure 2 Infrared spectrum of the product obtained after the reaction between A and methyl BCD.

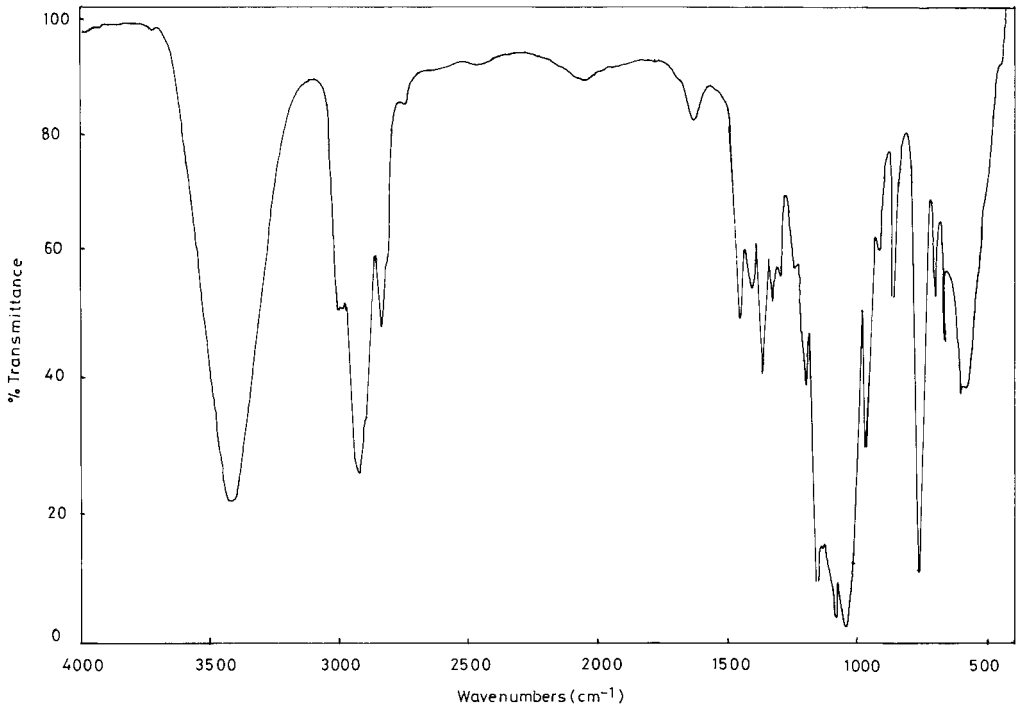

Figure 3 Infrared spectrum of methyl BCD.

Methyl BCD—NHCO—$(CH_2)_6$—
(product B)

NHCO—(HEMA) (1)

The polymer obtained after polymerizing the modified HEMA is found to be insoluble in all common organic solvents such as methanol, chloroform, tetrahydrofuran, and dimethylacetamide, indicating the feasibility of using the new polymer as a matrix for application such as in chromatography.

Table I summarizes the extent of the absorption of cholesterol by the polymer from methanol and hexane. The polymer absorbs a higher amount of cholesterol from methanol, indicating the strong influence of the medium on the interaction. Being a hydrophobic molecule, the solubility of cholesterol is lower in methanol than in hexane. The low solubility in methanol may be the driving factor for the enhanced absorption of cholesterol by the polymer.

Table II shows the uptake of testosterone by the polymer from methanol and hexane. Interestingly, the extent of interaction of testosterone with the polymer is just opposite to what is observed in the case of cholesterol. Again, it seems that the solubility factor is playing the major role in the absorption process. Testosterone is more polar compared to cholesterol and, hence, its solubility is greater in methanol than in hexane. The data summarized demonstrate the strong dependence of the medium on the interaction of the steroids with the methyl BCD polymer. A glance at the tables clearly shows that from methanol the extent of absorption of cholesterol is much higher than that of testosterone. The polymer has a higher affinity toward cholesterol than toward testosterone when the compounds are dissolved in methanol. This aspect is further demonstrated

Table I Extent of Absorption of Cholesterol by Methyl BCD Polymer

Medium in Which Cholesterol is Dissolved	Amount Absorbed by 100 mg Polymer
Hexane	2.85 ± 0.05 mg
Methanol	5.23 ± 0.02 mg

Table II Extent of Uptake of Testosterone by the Polymer

Medium	Amount Absorbed by 100 mg Polymer
Hexane	2.63 ± 0.03 mg
Methanol	1.89 ± 0.04 mg

Table III Absorption of Cholesterol and Testosterone from Their Mixture in Methanol

Compound	Extent of Absorption by 100 mg Polymer
Cholesterol	6.67 ± 0.05 mg
Testosterone	1.06 ± 0.03 mg

in Table III which depicts the extent of absorption of these steroids from a mixture of these two components in methanol. It is apparent that the polymer exhibits some degree of selectivity in its interaction toward cholesterol in the presence of testosterone. The amount of testosterone absorbed from the mixture is less than its quantity absorbed from methanol (see Table II). This variation in absorption of testosterone could be assigned to the higher affinity of methyl BCD toward cholesterol.

The strong influence of the polarity of the medium on the uptake of cholesterol is further demonstrated in Table IV. The polarity of methanol is further increased by adding water (traces). One interesting aspect is the considerable increase in the extent of uptake of cholesterol, which could be considered as due to the influence of the polarity on the selectivity in absorption. Further addition of water leads to the precipitation of cholesterol, and, hence, the possible improvement in selectivity in absorption could not be attempted. However, the results summarized here sufficiently point out the greater role of polarity of the medium in the selectivity in the absorption of cholesterol by the polymer.

The reusability of the polymer is demonstrated in Table V. The polymer after equilibrating with the cholesterol solution is extracted with chloroform and then placed in the cholesterol solution in methanol. The uptake of cholesterol is nearly equal in each cycle, indicating that the affinity of the polymer toward cholesterol is unaffected by the extraction process.

Table IV Uptake of Cholesterol and Testosterone by the Polymer from Methanol/Water Mixture

Compound	Absorption by 100 mg Polymer
Cholesterol	8.17 ± 0.06 mg
Testosterone	0.77 ± 0.03 mg

Table V Effect of Extraction on Absorption Capacity of the Polymer

Extraction Cycle	Absorption by 100 mg Polymer
0	5.23 ± 0.02 mg
1	5.01 ± 0.04 mg
2	5.33 ± 0.03 mg
3	5.18 ± 0.02 mg

CONCLUSIONS

A simple approach has been discussed to synthesize methyl BCD-based polymer. The polymer is stable and insoluble in organic and aqueous media. The polymer exhibits affinity toward steroids including cholesterol. The selectivity in absorption of cholesterol is largely determined by the polarity of the medium. It seems that the polymer could be tailored as an absorbent for cholesterol.

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